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Investigating Intra-individual Dietary Changes and Radiocarbon Ages Using High Resolution $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ Isotope Ratios and ^{14}C Ages Obtained From Dentine Increments

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INVESTIGATING INTRA-INDIVIDUAL DIETARY CHANGES AND ^{14}C AGES USING HIGH-RESOLUTION $\delta^{13}\text{C}$ AND $\delta^{15}\text{N}$ ISOTOPE RATIOS AND ^{14}C AGES OBTAINED FROM DENTINE INCREMENTS

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ABSTRACT. Ten medieval permanent teeth were subjected to incremental dentine sectioning and stable isotope analysis to investigate dietary changes in high resolution. In addition to this, eight increments were also selected for radiocarbon measurements to examine possible intra-individual age differences. Results reveal the cessation of weaning, various dietary profiles, and in some cases significantly different ^{14}C ages obtained from a single tooth. This case study illustrates how ^{14}C measurements can function as a proxy alongside the commonly used carbon and nitrogen stable isotope values to interpret the diet of past individuals.

INTRODUCTION

Multiproxy and multitissue analyses of increasing resolution are becoming the norm in stable isotopic research to gather as much information as possible from different life stages of the deceased (Müldner et al. 2011; Knudson et al. 2012; Lamb et al. 2012, 2014; Oelze et al. 2012; Touzeau et al. 2014). One way of obtaining high-resolution dietary information is the application of stable isotope analysis on incrementally sampled dentine sections (Beaumont et al. 2013b). Since tooth enamel and (most of the) dentine are formed during childhood (Hillson 1996, 2005), permanent dentition will therefore reflect information recorded during the juvenile period (Katzenberg 2008). As such, every adult individual will carry around his or her “inner child” in their teeth. This is an interesting notion, seeing as juveniles have been largely underrepresented in archaeological contexts due to a taphonomic bias. The lower degree of mineralization renders juvenile bones and teeth more susceptible to bone degradation (Manifold 2014). The incremental dentine method from Beaumont et al. (2013b) provides an excellent opportunity to incorporate childhood diet on a more regular basis in stable isotope studies.

The current case study is part of a larger, international project that aims to investigate human-coast environmental interaction in the Limfjord in Denmark. Part of this project will consist of stable isotope analysis on incremental dentine samples obtained from prehistoric (Neolithic to Viking Age) teeth from the Limfjord region. With this high-resolution method, it will be possible to zoom in further on a single individual, providing a more in-depth, personal dietary study of past individuals from this region. More importantly, it allows us to examine *changes* in diet. In addition to the stable isotope aspect, contemporary environmental data, absent in most stable isotope studies, will be obtained by performing a range of geochemical analyses on sediment cored from the Limfjord. This will hopefully provide a solid environmental setting to aid in our understanding of the stable isotopic record, and dietary changes especially, across the millennia.

While the prehistoric teeth await, this paper reports stable isotope results from a case study on Danish medieval teeth to examine this dentine sectioning method before applying it to the older and more valuable prehistoric specimens. In addition to the stable isotope analysis, several dentine sections were subjected to accelerator mass spectrometry (AMS) radiocarbon measurements to identify possible intratooth age differences. As such, the AMS ^{14}C measurements can be used as a proxy to help explain and interpret the stable isotope ratios.

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AALBORG MONASTERY AND CEMETERY

Aalborg is situated in the north of Jutland, Denmark, on the coast of the Limfjord (Figure 1). Further to the west, the water merges into the much larger water body of the Limfjord and into the Kattegat in the east. A Franciscan monastery was established in Aalborg around AD 1240, which fell out of use and was closed down around AD 1530. The monastic cemetery served as parish churchyard for Aalborg, while the monks were probably buried in adjacent nonexcavated parts of the monastic complex (Bergmann Møller 2000). In 1994–1995, parts of the monastery buildings and the churchyard were excavated, bringing forth some 500 skeletons (Lynnerup and Møllerup 2000). By far, most were adults (~40 subadults), with a 60:40 male to female ratio. The age-at-death for both females and males resembled other contemporary, Danish medieval cemeteries. In terms of general health, the skeletons may reflect an urban, better-off population, rather than a rural population (Lynnerup et al. 2002). This was also reflected in calculated statures, which indicated slightly higher statures than found for contemporary, rural skeletons. No skeletons displayed overt lesions indicative of malnutrition or diseases due to lack of vitamins, etc. (Lynnerup and Møllerup 2000).

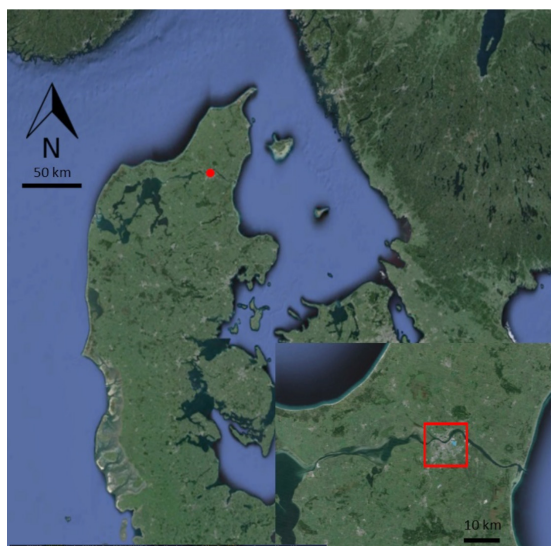


Figure 1 Google Maps image of the Jutland Peninsula in Denmark with the city of Aalborg marked in red in the inset.

DIFFICULTIES IN STABLE ISOTOPE DATA INTERPRETATION

Processes of fractionation producing a shift in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope ratios can reveal information about the consumed diet and an individual's trophic position. In northern Europe, carbon stable isotopes are useful in separating terrestrial from marine dietary inputs (Schoeninger and DeNiro 1984), whereas nitrogen stable isotopes can be used to determine the average trophic level of protein consumed (Price et al. 1985; Schoeller 1999). Protein sources can be either terrestrial, freshwater, or marine in origin.

However, as carbon stable isotope values can differ regionally (van Klinken et al. 2000), it is important to use comparative data from nearby regions. Additionally, consumption of marine sources can be masked in terms of $\delta^{13}\text{C}$ values in low-protein diets (Hedges 2004). These diets can lead to "scrambled routing" of carbon from multiple macronutrients, i.e. protein, carbohydrates, and lipids, which can result in depleted $\delta^{13}\text{C}$ values. Difficulties similarly exist in the interpretation of nitrogen

stable isotope ratios, as these can be influenced by factors other than diet (Hedges and Reynard 2007). Animals feeding on plants grown on soils enriched in $\delta^{15}\text{N}$ values due to oceanic salt spray (Virginia and Delwiche 1982; Heaton 1987), high salt content (Heaton 1987; Britton et al. 2008), or fertilizers (van Klinken et al. 2000) can consequently display elevated $\delta^{15}\text{N}$ values in their tissues. As these elevated nitrogen stable isotope values are carried up the food chain, they can eventually influence human $\delta^{15}\text{N}$ values as well (van Klinken et al. 2000).

In addition to the environmental factors, nitrogen stable isotope ratios can be influenced by internal factors, such as physiology, nutrition, health, and protein stress. Though not completely understood, several studies have made important contributions towards an understanding of the complex interactions between these factors. During periods of bodily equilibrium, ingested nitrogen is first subjected to processes of transamination and deamination before being incorporated into tissues (Reitsema 2013). As these processes introduce fractionation, the formed tissues are enriched in ^{15}N , while waste products (e.g. urea) contain more ^{14}N (Steele and Daniel 1978; Reitsema 2013). During periods of bad health/illness and/or nutritional stress, insufficient amounts of protein are ingested, forcing the body to catabolize its own tissues. Nitrogen in these tissues is fractionated for the second time, resulting in even higher $\delta^{15}\text{N}$ values (Hobson et al. 1993; Fuller et al. 2005). On the other hand, during periods of positive nitrogen balance, e.g. rapid growth (Williams et al. 2007) or pregnancy (Fuller et al. 2004), nitrogen may be routed directly to tissues, thereby skipping processes of transamination and deamination and, as a result, also fractionation. Direct routing would therefore produce tissues with relatively depleted $\delta^{15}\text{N}$ values (Reitsema 2013). However, minimal and even moderate protein stress might not be recorded in the $\delta^{15}\text{N}$ values due to certain threshold values (Kempster et al. 2007). This is illustrated by elevated $\delta^{15}\text{N}$ values in hair samples from anorexic patients (Mekota et al. 2006), whereas normal $\delta^{15}\text{N}$ values were obtained from bulimic patients (Hatch et al. 2006). This suggests that bulimia, though a severe eating disorder, is not severe enough to result in elevated $\delta^{15}\text{N}$ values (Reitsema 2013). It is important to keep in mind that most of these studies are performed on human hair, which has a much higher turnover rate than bone. As femoral bone remodeling takes a decade or more for complete turnover (Hedges et al. 2007), the stable isotopic values are an average of that time. Any enrichment in $\delta^{15}\text{N}$ values corresponding to periods of protein stress might have been evened out and might not be visible in the bone's $\delta^{15}\text{N}$ ratios. High-resolution incremental dentine samples could record some of these physiological effects, given that they are severe enough to produce deviant $\delta^{15}\text{N}$ values. Whether any physiologically induced deviant $\delta^{15}\text{N}$ ratios are retained or averaged out depends on the formation time of the corresponding dentine increment. Generally, variation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios is expected to be mostly related to dietary changes.

Weaning

A newborn baby's nitrogen stable isotope signal will be similar to that of its mother (Richards et al. 2002), although the $\delta^{15}\text{N}$ values will become elevated over time, indicating a breastfeeding signal (Fogel et al. 1989; Fuller et al. 2006b). The nitrogen stable isotope ratios become elevated, as the infant "eats" its mother and is therefore one trophic level above her (Fogel et al. 1989; Schoeninger and DeNiro 1984). No increased $\delta^{15}\text{N}$ values have been detected for solely bottle-fed babies (Fuller et al. 2006b). Over time, other food is fed to the infant alongside the maternal milk, until the juvenile has made the complete switch from milk to non-milk foods and breastfeeding has ceased, which is also referred to as the weaning age (Katzenberg et al. 1996; Herring et al. 1998). During the weaning process, referring to the period between 100% breastfeeding and the cessation of breastfeeding, varying quantities of maternal milk and other foods are consumed (Dettwyler and Fishman 1992; Katzenberg et al. 1996; Herring et al. 1998), causing the juvenile's tissue $\delta^{15}\text{N}$ values to come down due to a decrease in maternal milk intake (Fuller et al. 2006b).

The weaning age can differ regionally, chronologically, and culturally. Richards et al. (2002) found that weaning had ceased around the age of 2 in medieval individuals from Wharram Percy in the UK. A similar weaning age was observed by Burt (2013) in juveniles from medieval Fishergate House, also in the UK, while Fuller et al. (2006a) found that weaning had ceased between the ages of 3–4 in Late Roman individuals from the UK.

Dentine Formation

Human tooth formation is a complex process that starts in utero and is finalized in the early twenties (AlQahtani et al. 2010). Human enamel becomes avascular after completion, rendering enamel unable to remodel after formation. This is slightly different for dentine, which can be subdivided into primary, secondary, and tertiary dentine. Primary dentine, consisting of a few μm of mantle dentine with the rest being circumpulpal dentine, comprises the bulk of the dentine and is the targeted tissue here. Primary dentine is formed before and during eruption of the tooth (Piesco 2002a; Garg and Garg 2013; Nanci 2013). After completion, dental tubules remain present, in contrast to enamel, allowing the deposition of secondary dentine inside the pulp chamber during life (Hillson 1996). Tertiary (or reparative) dentine is formed in response to caries, attrition, and trauma (Piesco 2002a; Garg and Garg 2013). The degree of secondary and tertiary dentine formation depends on physiological and pathological stimuli (Garg and Garg 2013; Nanci 2013). By avoiding damaged or decayed teeth and removing secondary dentine from the pulp chamber, primary dentine can be obtained, which does not remodel during life (Piesco 2002a; Hillson 2005) and therefore contains isotopic values representing the childhood period.

Human primary dentine begins to form in what later becomes the crown and moves away from the dentinal enamel junction (Figure 2). The dentine is laid down in “sleeves” or curves around the pulp chamber (Hillson 1996, 2005; Piesco 2002a). This makes sampling the actual increments quite difficult as the increments curve around the pulp chamber and down the root. As such, horizontal sampling will always result in the average isotopic value of multiple “sleeves,” although these average values still represent a sequential chronological order (Beaumont et al. 2013b). Most teeth used in this study are second molars, which are formed over a timespan of 13 yr, from 2.5 ± 0.5 to 15.5 ± 0.5 yr of age. The second premolar in this data set had a formation time of 12 yr, from 2.5 ± 0.5 to 14.5 ± 0.5 yr of age, while the third molar had a formation time of 15 yr, from 8.5 ± 0.5 to 23.5 ± 0.5 yr of age (AlQahtani et al. 2010).

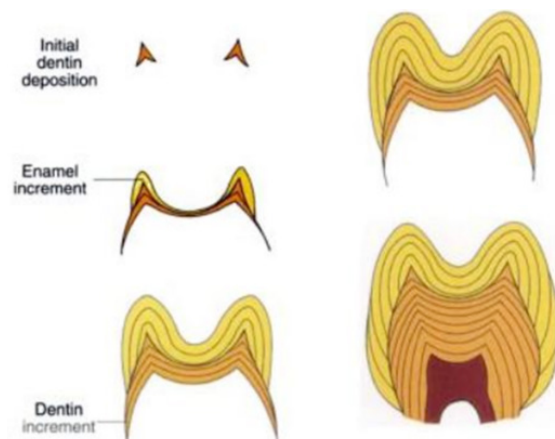


Figure 2 Enamel and dentine formation begins at the crown. This image clearly illustrates how layers of dentine are deposited, curving around the pulp chamber. Image source with courtesy of Georg Thieme Verlag KG (Piesco and Avery 2002b:91).

Material and Methodology

The teeth used in this study are stray finds originating from the excavation of a medieval church in Aalborg in northern Denmark and were available for analysis of incremental dentine. Apical closure indicated that all teeth were fully developed. Table 1 shows the information of each tooth in terms of museum sample and code numbers. The number of sections taken from each tooth is noted here as well. Unfortunately, the absence of contextual information inhibits comparison of samples in terms of gender, age, burial practice, and corresponding adult dietary information based on the individuals' bone collagen stable isotope values. Additionally, faunal bone material to provide an isotopic background signal is lacking, which is normally required for a correct interpretation of the human isotopic results. However, in this study we can still investigate intra-individual dietary changes during childhood using high-resolution data and subsequently highlight the methodological possibilities. Additionally, eight sections from three individuals (AIF, Kirke, and DA) with varying dietary patterns were selected for AMS ^{14}C measurements to identify possible intratooth age differences, which could help explain the stable isotope ratios.

Table 1 Overview of the information on the medieval teeth.

Sample	Code	Tooth	Side	Nr of sections
AIF (2:37)	+7	maxillary 2nd molar	Left	18
Løsfund rasn (kasse 1) AHM 2481x1123 (1:69)	+7	maxillary 2nd molar	Left	16
Lag i kirken (2:45)	+7	maxillary 2nd molar	Left	16
DA (2:42)	-8	mandibular 3rd molar	Left	16
Kirke (2:63)	7-	mandibular 2nd molar	Right	18
Fy, DA (2:15)	-7	mandibular 2nd molar	Left	16
PAL CF AQ+R (2:62)	5-	mandibular 2nd premolar	Right	16
Felt AZA BCO (1:3)	7+	maxillary 2nd molar	Right	15
Kloakgrv (2:45)	+7	maxillary 2nd molar	Left	12
Løs knogler BG AHM 2481x1123 (1:59)	+7	maxillary 2nd molar	Left	18

Incremental Dentine

The last couple of years have seen great improvement in the microsampling of tooth dentine (Fuller et al. 2003; Kirsanow et al. 2008; Eerkens et al. 2011; Beaumont et al. 2013a,b; Eerkens and Bartelink 2013). For teeth in this study, the second method proposed in Beaumont et al. (2013b) was applied, as this is suggested for well-preserved teeth and less material is lost in the process. The first method involves imbedding teeth in dental plaster before cutting them into slices, resulting in slightly thicker sections, lower resolution, and more of the sample is lost in the process. From each tooth, the longest root and associated part of the crown was cut using a Dremel fitted with a diamond-coated cutting blade in a fume cupboard. As each tooth is formed within a certain time-span, a longer transect from root to crown allows more increments to be sampled, thus increasing the resolution. Each cut tooth was stripped of its enamel using the Dremel and cleaned by gently abrading the surface with the dremel. Any secondary dentine, which is deposited inside the pulp chamber during adulthood, was removed after demineralization, as this can present very different stable isotopic values.

Collagen Extraction

A modified Longin protocol (Longin 1971), similar to Brock et al. (2010) and altered by Beaumont et al. (2013b), was followed for collagen extraction. The entire tooth was demineralized in 0.6M HCl (aq.) acid, during which the acid was renewed several times to ensure demineralization was

complete. Tooth sectioning took place using a scalpel alongside a ruler to sample 1-mm increments. Weak HCl (aq.) acid (pH 3) was added to gelatinize the increments at a temperature of $\sim 70^{\circ}\text{C}$ for 24 hr. After this, samples were frozen, lyophilized, and sampled in tin cups for carbon and nitrogen stable isotope analysis using the Thermo Delta V IRMS with Flash EA at the $^{14}\text{CHRONO}$ Centre in Belfast. To ensure high-quality results were obtained, all samples were required to display collagen yields higher than 1% (van Klinken 1999), atomic C/N ratios between 2.9–3.6 (DeNiro 1985), and %C and %N between 15.3–47% and 5.5–17.3%, respectively (Ambrose 1990). The standard error on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements is 0.22‰ and 0.15‰, respectively, which is added to the results in Figure 3 as well.

Radiocarbon Dating

Eight increments from three teeth were selected for AMS ^{14}C measurements based on their varying dietary patterns with visibly diverging $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios. About 2.5–3 mg of extracted collagen was loaded in Pyrex glass tubes with excess CuO and silver ribbon, after which the tubes were sealed and samples were combusted at 560°C . The produced CO_2 was reduced to graphite using a hydrogen reduction method with iron as a catalyst. Pressed targets were analyzed alongside oxalic acid standards and background samples in the NEC compact model 0.5MV AMS at the $^{14}\text{CHRONO}$ Centre in Belfast. ^{14}C ages are calculated from $F^{14}\text{C}$ (Reimer et al. 2004), which is corrected for background and for isotopic fractionation using $^{13}\text{C}/^{12}\text{C}$ measured by AMS that accounts for both natural and machine isotopic fractionation. A complete description of the ^{14}C age and error calculation is given in Reimer et al. (2015). Briefly, errors on the ^{14}C measurements are estimated from the maximum of the variance of seven 2-min runs and the propagated error, which includes the uncertainty in the $^{14}\text{C}/^{12}\text{C}$ ratio for each run of the sample, standard, and background. To account for variability in our pretreatment procedure, an error multiplier is calculated from the ratio of the long-term variance and the average of a secondary standard of the same material type. The error in the $F^{14}\text{C}$ measurements is multiplied by this ratio, which is 1.3 for bone collagen.

RESULTS

Results are graphically reported in Figure 3 (see the online [Appendix](#) for the complete table). The averages of all increments were $-19.57 \pm 0.65\text{‰}$ and $11.82 \pm 1.16\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Most teeth yielded long enough root to crown transects for 15 or more 1-mm increments, although one tooth only produced 12 increments (Kloakgrv). With most teeth yielding 15 to 18 increments of second molars, which have a formation time of approximately 13 yr, intra-annual resolution of the stable isotope results was reached. Since only one root and the corresponding crown part were cut, about half a tooth was destroyed, leaving the remaining half intact and available for future work, e.g. aDNA analysis. Some increments were low yielding and not enough collagen was obtained for analysis (PAL CF AQ+R 1, 14, and 16), while others produced enough for duplicate measurements. All samples produced acceptable atomic C/N ratios, ranging from 3.11 to 3.39. Most teeth present clear correlations between the carbon and nitrogen stable isotope ratios, although three teeth, AIF, Felt AZA BCO, and Løsfund rasn AHM 2481x1123, presented the highest correlations of $R^2 = 0.85$, 0.82, and 0.59, respectively. Dentine increments were selected for ^{14}C dating based on their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Sufficient collagen was available for AMS ^{14}C measurement of most single increments, although collagen from two increments had to be combined in three cases (DA increment A, AIF increment C) (Figure 3).

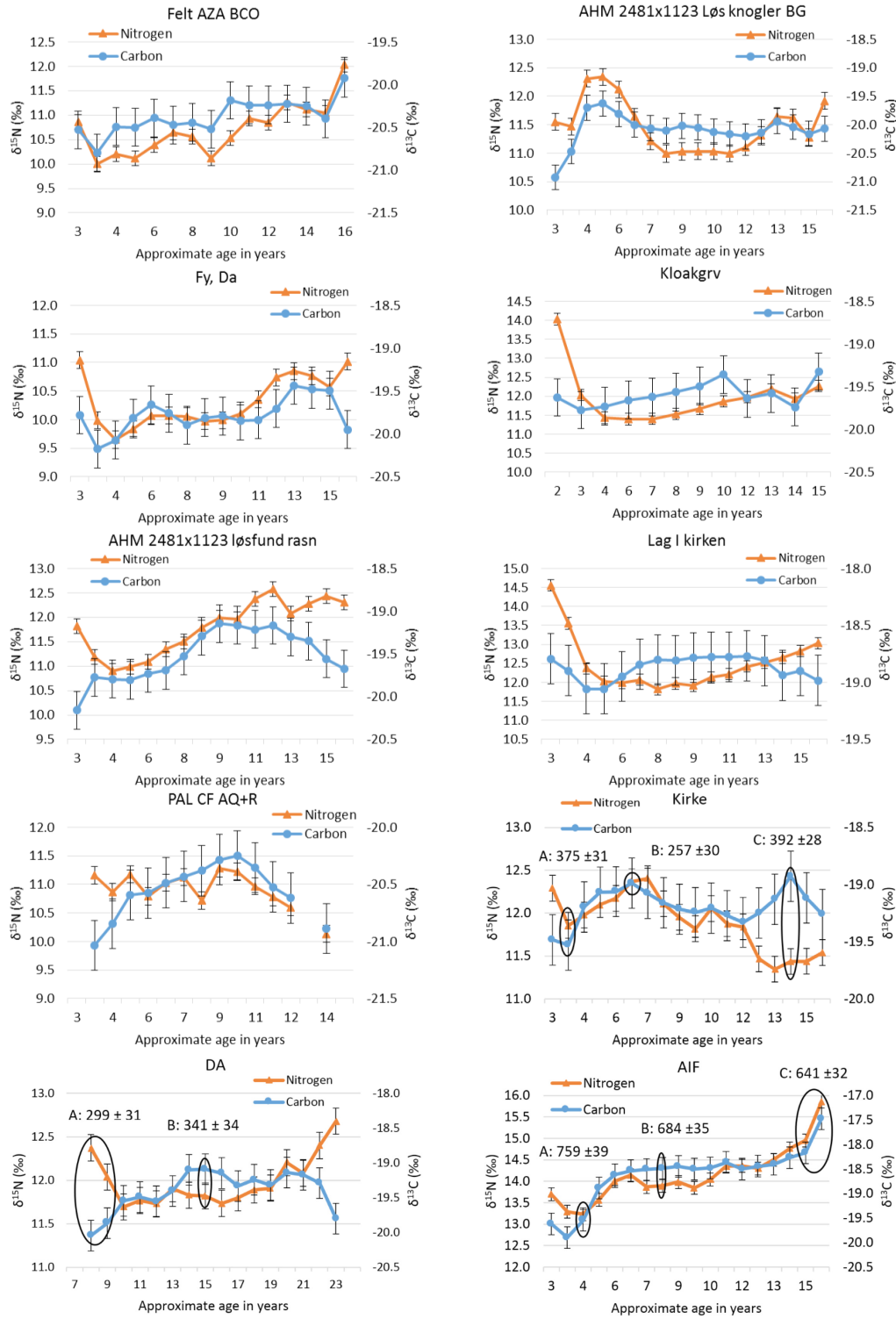


Figure 3 Carbon and nitrogen stable isotope profiles from the 10 teeth. The ^{14}C measurements are displayed in the last three teeth (Kirke, DA, and AIF).

DISCUSSION

Carbon and nitrogen stable isotope ratios appear overall well coupled. There is no clear evidence for physiological effects on the stable isotope data, as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios are both dynamic and changes between them appear diet related. Most profiles display a certain trend, beginning with an initial drop in stable isotope values on the left side of the profile, displaying the lowest stable isotope values at this point, followed by the gradual elevation in stable isotope values. However, not all individuals follow this trend exactly. Three individuals (PAL CF AQ+R, Kirke and AHM 2481x1123 Løs knogler BG) display a short-term elevation in stable isotope values, followed by a downward trend. Also noteworthy are three samples (AHM 2481 x 1123 losfund rasn, DA and PAL CF AQ+R) that show decreasing $\delta^{15}\text{N}$ values and increasing $\delta^{13}\text{C}$ values during the weaning phase.

Weaning

Most of the teeth used in this study are second molars, which initiate formation around 2.5 yr of age. As weaning usually starts well before that age, only remnants of the weaning process are visible in the earliest forming dentine of second molars. Other foods introduced to the infant during weaning are most likely easily digestible, low trophic level food sources, resulting in decreased $\delta^{15}\text{N}$ ratios, as is seen in individuals from Wharram Percy (Mays et al. 2002; Richards et al. 2002; Fuller et al. 2003). This continues until the infant is completely weaned and adjusted to foods other than maternal milk, resulting in the lowest stable isotopic values in most profiles. After this, higher trophic level foods are fed to the juvenile, which subsequently causes stable isotope ratios to rise again. The stable isotope values would usually level around the adult stable isotopic values (Burt 2013), although we cannot investigate this due to absence of corresponding bone collagen. Still, our profiles suggest weaning of these individuals ceased between 3–4 yr of age, which is similar to the weaning age of Late Roman individuals in Britain (Fuller et al. 2006a), yet relatively late compared to the weaning age of 2 yr observed in medieval Wharram Percy (Mays et al. 2002; Richards et al. 2002; Fuller et al. 2003), medieval Fishergate House (Burt 2013), and the assumed weaning age for medieval Europe in general (Fildes 1995). The food introduced to most Danish individuals during weaning probably consisted of low trophic level, plant-based food, indicated by the low $\delta^{13}\text{C}$ ratios in most profiles (about -20‰ and 21‰), although there are a few exceptions with slightly higher $\delta^{13}\text{C}$ ratios, e.g. Lag i kirken (19‰) and Kirke (-19.5‰).

Richards et al. (2002) found that middle childhood diet, roughly between 4–8 yr of age, displayed somewhat lower nitrogen stable isotope values compared to later childhood and adulthood. The authors argue that this is most likely not due to periods of intense growth but more a matter of diet; the diet of these young children was probably more plant-based. This is in correspondence with our data, as most profiles display lower stable isotope values in the period directly after the weaning compared to later in childhood.

Childhood Diet

This section refers to the period after the cessation of weaning. Nitrogen stable isotope values of 11‰ and 12‰ are not uncommon in our profiles, suggesting these individuals consumed considerable amounts of higher trophic level protein during their young lives. Corresponding $\delta^{13}\text{C}$ values ranging roughly between 20‰ and -19‰ suggest this is probably a marine effect. Variation in profiles with more elevated $\delta^{15}\text{N}$ values could be attributed to consumption of freshwater fish, pigs fed on fish scraps, or terrestrial animals fed on soils enriched in $\delta^{15}\text{N}$ values. Additionally, marine protein consumption could be less pronounced in the $\delta^{13}\text{C}$ values in low protein diets (Hedges 2004).

Some medieval faunal and human stable isotope data from Jutland are available for comparison

from Yoder (2010) (Table 2). Average stable isotope results from our study ($11.82 \pm 1.16\text{‰}$ and $-19.57 \pm 0.65\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively) are similar to the bone collagen values obtained by Yoder (2010) from three medieval sites in Jutland. Since Aalborg was a coastal town, fresh fish was readily available from the Limfjord's waters, although dried stockfish and salted clipfish from markets probably also formed part of the diet (Lauring 1963; Enghoff 1996; Barrett et al. 2008; Yoder 2010). The terrestrial domestic herbivores reveal somewhat elevated nitrogen stable isotope values compared to the elk and deer, which could be induced by consumption of plants enriched in $\delta^{15}\text{N}$ due to salt spray, saline soils (Virginia and Delwiche 1982; Heaton 1987; Britton et al. 2008), or manuring (van Klinken et al. 2000). The current sample size is too small to draw any conclusions on the feeding behavior of the animals. Although it remains difficult to identify the underlying causes for the absolute stable isotope values of the Aalborg individuals without abundant contemporaneous animal stable isotope data, it is possible to examine potential causes responsible for any profile patterns. One individual (AIF) shows continuous elevation in both isotopes up to 16‰ in $\delta^{15}\text{N}$ values and -17.5‰ in $\delta^{13}\text{C}$ values, which is most likely induced by marine protein consumption. This child's nitrogen stable isotope values never dropped below 13‰ , which is remarkably high compared to other individuals. This possibly indicates marine protein was present in the mother's diet and the subsequent weaning food as well. Most profiles show positive correlations between carbon and nitrogen, although there are some exceptions. As marine food sources produce an enrichment in both isotope values, this can be easily detected (AIF). However, an increase in nitrogen stable isotope values combined with a drop in carbon stable isotope values would suggest a switch to more terrestrial-based protein (DA). Likewise, increasing $\delta^{13}\text{C}$ ratios combined with decreasing $\delta^{15}\text{N}$ ratios would therefore suggest higher proportions of low trophic level marine food sources in the diet (Kirke), possibly shellfish. Again, this remains speculative due to the absence of an isotopic baseline for the region based on animal stable isotope ratios.

Table 2 Animal and human stable isotope data from Jutland (Yoder 2010).

Sample	<i>n</i>	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Humans Øm Kloster	55	11.64 ± 0.77	-19.86 ± 0.42
Humans St. Mikkel	45	12.39 ± 0.71	-19.24 ± 0.42
Humans Ribe	54	12.85 ± 0.91	-19.19 ± 0.54
Elk Øm Kloster	1	3.55	-21.43
Deer Øm Kloster	1	1.99	-21.53
Pig Øm Kloster	1	6.03	-21.28
Goat Øm Kloster	1	7.76	-21.81
Chicken Øm Kloster	1	10.34	-22.22
Cow Øm Kloster	1	6.34	-21.53

Radiocarbon Ages

We were interested to examine possible ^{14}C age differences in increments where the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios seemed to diverge and indicate a shift in diet. Two sections from tooth DA were selected to investigate the combination of low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ ratios around 8 yr of age, compared to the opposite trend around 15 yr of age (Figure 3). The ^{14}C ages from these two increments were statistically the same.

Increments B and C from tooth Kirke with the same $\delta^{13}\text{C}$ ratio (-18.9‰) but different $\delta^{15}\text{N}$ ratios (12.3‰ and 11.4‰) produced ^{14}C ages of 257 ± 30 and 392 ± 28 , respectively. The increased ^{14}C age in conjunction with the lower $\delta^{15}\text{N}$ ratio in C could be explained by increased proportions of

low trophic level marine food in the diet, such as shellfish. The ^{14}C age of B is significantly different from A ($\chi^2(0.05) = 3.84$, $T' = 7.48$) and C ($\chi^2(0.05) = 3.84$, $T' = 10.82$).

Two (A and C) of the three increments from AIF yielded significantly different ^{14}C ages ($\chi^2(0.05) = 3.84$, $T' = 5.47$). Despite the high isotopic values in increment C ($\delta^{13}\text{C}$ ratio = -18% and $\delta^{15}\text{N}$ ratio = 15.5, note that the collagen from two increments was combined), the ^{14}C age is younger than A. Strong coupling and elevation of both stable isotope values seems to point to increased marine protein in the diet, although we would then expect an increase in age as well. Since freshwater fish consumption is known to produce significant ^{14}C reservoir offsets, small proportions of freshwater fish could be responsible for this high ^{14}C age combined with relatively depleted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Fernandes et al. 2015).

Table 3 ^{14}C ages and $F^{14}\text{C}$ of the selected samples.

UBA lab nr	Sample ID	^{14}C age BP	$F^{14}\text{C}$
UBA-25833	AIF_1	759 ± 39	0.9098 ± 0.0044
UBA-25834	AIF_2	684 ± 35	0.9184 ± 0.004
UBA-25835	AIF_3	641 ± 32	0.9233 ± 0.0036
UBA-25836	DA_1	299 ± 31	0.9634 ± 0.0037
UBA-25837	DA_2	341 ± 34	0.9584 ± 0.004
UBA-25838	Kirke_1	375 ± 31	0.9544 ± 0.0037
UBA-25839	Kirke_2	257 ± 30	0.9685 ± 0.0036
UBA-25842	Kirke_3	392 ± 28	0.9524 ± 0.0034

This study showed that dentine increments from a single tooth with varying stable isotope ratios may differ in their ^{14}C ages. Even though in some cases significantly different ^{14}C ages from a single individual were obtained (Kirke and AIF), it should be stressed that the sample size of the ^{14}C measured specimens in this study is small and the ^{14}C aspect needs to be studied more in depth. Nevertheless, due to the complex interplay between food source and quantity, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios should be carefully examined in conjunction with ^{14}C ages. Tooth dentine is a material commonly used for ^{14}C dating. While whole teeth will produce a ^{14}C age averaged from all sections together, the use of partial teeth (e.g. dentine roots), or a combination of all forms of dentine (primary, secondary, and tertiary), might produce a deviant ^{14}C age (Cook et al. 2006). The incremental sectioning method from Beaumont et al. (2013b) might be useful in forensic cases that aim at providing a date of birth. This has been done on tooth enamel (Calcagnile et al. 2013), although the extent of a possible reservoir effect can be difficult to establish without the corresponding stable isotope data (as suggested by Cook et al. 2006).

CONCLUSION

This case study revealed that stable isotope analysis on incremental dentine worked very well on these samples. The well-preserved nature of the medieval teeth provided an excellent opportunity to explore the methodology and show that high-resolution dietary information can be obtained. This is particularly useful when examining rapid dietary changes, which would remain invisible using bone collagen stable isotope data.

More Danish teeth from various archaeological time periods will be analyzed for their $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ ratios using this sectioning methodology, which could be a useful tool in untangling the extent of marine and freshwater reservoir effects, especially when sulfur stable isotope ratios are included. The enamel was not used in this study due to the focus on incremental dentine, although

this could be analyzed alongside the dentine in future studies, for example, in strontium or oxygen isotope-based studies. As such, half a tooth can yield an incredible amount of information on an individual's past life, using both the tooth's mineral and protein fraction.

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